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Genetics

Mobile inverted-repeat elements of the *Tourist* family are associated with the genes of many cereal grasses

(maize/sorghum/rice/transposable elements/genome evolution)

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ABSTRACT ***Tourist*** **was originally described as a 128-bp insertion mutation in the maize *wx-B2* allele. Subsequent analysis revealed that *Tourist* elements are in the introns or flanking sequences of 11 maize genes and a single barley gene. In this study we report that *Tourist* elements are frequently associated with the wild-type genes of two other grasses, rice and sorghum. Six of 35 rice and 5 of 8 sorghum complete gene sequences reported to date contain *Tourist* elements. Furthermore, 11 additional maize genes have been found to contain *Tourist* elements, bringing the current total of elements associated with maize genes to 23. Sequence comparison of *Tourist* elements has led to the identification of four subfamilies, designated *A–D*. Evidence is presented for the recent mobility of elements in three of these subfamilies and in three of the four grass species. These data suggest that *Tourist* elements are highly repetitive in the genomes of some and perhaps all members of the grasses.**

Repetitive DNA accounts for a large fraction of the genomes of most higher eukaryotes (1). It has been suggested that mobile DNA elements make up most of the dispersed component of repetitive DNA (2–5). In some species this prediction has been borne out. The *Alu* family of retroposons accounts for at least 5% of the genome of primates (6). *Alu* elements have been associated with insertion mutations and are also found in the introns and flanking sequences of wild-type genes. The 57-kb human hypoxanthine phosphoribosyltransferase gene, for example, was found to contain 49 *Alu* elements (7).

Plants harbor vast amounts of repetitive DNA resulting in genomes that are, on average, larger than the genomes of most animals (8). Although many plant transposable elements have been identified, virtually all are present in <100 copies per haploid genome (refs. 9–11, and references therein). Only a few elements, such as *del2* (12) and *Tourist* (13), account for a significant fraction of dispersed repetitive DNA. *del2* is a 4.5-kb non-long-terminal-repeat (LTR) retrotransposon that was estimated to make up ≈4% of the unusually large genome of *Lilium speciosum* (12).

Tourist was first identified as a 128-bp insertion in the *wx-B2* mutant of maize (13). This insertion mutation led to the discovery of a large family of related elements present in 1 barley and 11 maize genes. The *Tourist* family of elements is characterized by its small size, terminal inverted repeats (TIRs), 3-bp target site duplication (usually 5′-TAA-3′), and potential to form a hairpin structure. The identification of a *Tourist* element in a single barley gene prompted us to perform a more rigorous search for elements in other plant species. Here we report the identification of 25 additional *Tourist* elements that are in the introns or flanking sequences of wild-type genes from maize, barley, rice, and sorghum. Furthermore, we have used PCR analysis to demonstrate the recent mobility of *Tourist* elements in rice and sorghum.

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MATERIALS AND METHODS

Plant Material. Sorghum germ plasm was obtained from the USDA-ARS Southern Regional Plant Station at Griffin, Georgia, and *Oryza sativa* germ plasm was from G. Khush (International Rice Research Institute, Philippines). Sorghum and rice genomic DNAs were isolated as indicated by Dellaporta *et al.* (14). Genomic DNAs of other *Oryza* species were obtained from G. Kochert (University of Georgia, Athens).

DNA Sequence Analysis. Data base searches and sequence analyses were performed using the computer program suites of IntelliGenetics (version 5.4; IntelliGenetics) and GCG (version 7.0) (15) accessed through the BioScience Computing Resource at the University of Georgia. Data base searches using the algorithm BLAST (16) were performed via electronic mail to the National Center for Biotechnology Information at the National Library of Medicine (March 1993).

DNA Amplification. The following oligonucleotides were synthesized corresponding to sequences of the sorghum phospho*enol*pyruvate carboxylase (PEPC) (17) and rice phytochrome 18 (PHY18) (18) genes. Nucleotide positions relative to the start of translation are given in parentheses. PEPC2159S, 5′-GTTCTGTTCTTGGATGGGTG-3′ (nt 2159–2178); PEPC3829A, 5′-TTGCAGCGCCACATAGAGAG-3′ (nt 3829–3809); PHY183936S, 5′-CTGGCATGTCAACCTTCATC-3′ (nt 3936–3955); PHY184808A, 5′-CTTCAGTCTGCAAGTACGTCC-3′ (nt 4808–4788).

PCR was conducted as described (13). The PCR mixture consisted of 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, all four dNTPs (each at 200 μM), 70 ng of each primer, 200 ng of genomic DNA, and 2.5 units of *Thermus aquaticus* DNA polymerase (Perkin–Elmer) in a total volume of 100 μl. A step program of 1 min at 95°C, 2 min at 60°C, and 3 min at 72°C was repeated 40 times followed by a final extension at 72°C for 10 min. DNA fragments containing the fourth intron of the sorghum PEPC gene were amplified from sorghum genomic DNA using the primers PEPC2159S and PEPC3829A, blunt-ended, and cloned into pUC119 (19) digested with *Sma* I. Likewise, DNA fragments containing the fifth intron of the rice phytochrome 18 gene were amplified from rice genomic DNA using the primers PHY183936S and PHY184808A and subsequently cloned. Double-stranded templates were sequenced using a Sequenase kit (United States Biochemical) as directed by the manufacturer.

Abbreviations: TIR, terminal inverted repeat; PEPC, phospo*enol*pyruvate carboxylase; LTR, long terminal repeat.

RESULTS

***Tourist* Elements in Maize, Sorghum, Rice, and Barley.** To identify *Tourist* elements in the complete GenBank (version 75.0) and EMBL (version 34.0) data bases, a consensus TIR, based upon previously described maize and barley *Tourist* TIRs, was used as a query sequence with the search programs FASTA (GCG), FASTDB (IntelliGenetics), and BLAST (NCBI). Several additional *Tourist* elements identified in this way were then used as query sequences for further searches. The sequences summarized in Table 1 have been classified as *Tourist* elements because they share other features besides TIR similarity. Like the previously reported elements, most of these elements are short (113–343 bp), have significant potential to form DNA secondary structure, and are flanked by 3-bp direct repeats. In most cases, this direct repeat is 5′-TAA-3′, indicating a strong target site sequence preference (Table 1). All of the *Tourist* elements identified in our survey were associated with normal/wild-type genomic gene sequences.

When the elements in Table 1 are combined with the 11 elements reported in the prior study, it becomes evident that *Tourist* elements are a major component of the genomes of several grasses. To date, 23 *Tourist* elements have been found to be associated with maize genes, representing about one-third of the genomic maize gene sequences submitted to the GenBank and EMBL data bases (13). Similarly, 6 of 35 submitted genomic rice gene sequences, 5 of 8 submitted genomic sorghum gene sequences, and 2 of 40 submitted genomic barley gene sequences have at least one *Tourist* element in introns or flanking regions (only one allele for each locus was counted).

Four Subfamilies of *Tourist* Elements. All of the *Tourist* elements described to date can be classified into four subfamilies based on their length and/or conserved sequence domains. These features are summarized in Fig. 1. *Tourist A* members are small (133 bp on average) and have 5′-GGATT-3′ repeats that are not present in significant numbers in the other subfamilies. All of the elements described in a previous report belong to the *Tourist A* subfamily (13). *Tourist B* and *C* members range in sequence similarity from 46 to 95% (data not shown), resulting from three shared regions besides those held in common by all *Tourist* elements: (*i*) a conserved internal region (domain I), (*ii*) a subterminal poly(A)·poly(T) tract, and (*iii*) one copy of the sequence 5′-TCACATCGAAT-3′ located 39–50 bp from one terminus (box I) (Fig. 1 *A* and *B*). *Tourist C* elements are longer than *Tourist B* elements because they contain an additional domain, designated I′, which is like domain I (≈70% sequence similarity) but is in the inverse orientation. *Tourist D* members differ from other subfamilies by their

Table 1. *Tourist* elements found in the GenBank (version 75.0) and EMBL (version 34.0) data bases

Tourist name	Query	Definition	Position	Element size, bp	% AT	$\Delta G°$, kcal/mol	3-bp flanking host sequence	Ref.
Maize								
Zm13 (A)	*Zm6*	*Cat3* (4L)	5′ (−171)	137	53	−58.3	TAA TAA	20
Zm14 (A)	*Zm3*	Metallothionein	5′ (−421)	133	65	−37.3	GGA ATT	21
Zm15 (D)	*Os1*	Protein kinase	5′ (−935)	240	58	−74.8	TAA TAA	22
Zm16 (D)	*Zm15*	*Bz1* (9S)	3′ (+1852)	38*	ND	ND	TAA —	23
Zm17 (D)	*Zm15*	*P* (1S)	in 2 (+2385)	113	73	−28.2	CTA TAA	24
Zm18 (D)	*Zm20*	Oleosin	3′ (+1068)	299	67	−67.8	TAA TAA	25
Zm19 (D)	*Zm17*	Polyubiquitin	5′ (−1468)	159	82	−49.6	TAA TAA	26
Zm20 (D)	*Zm17*	*Adh1-1C*^{m} (1S)	3′ (+3642)	189	74	−84.6	TAA TAA	27
Zm21 (D)	*Zm17*	*Cat3* (4L)	in 2 (+2123)	51*	ND	ND	TAA —	20
Zm22 (A)	nl	*Shrunken-1* (9S)	in 7 (+2941)	139	63	−36.3	TAA TAA	28
Zm23 (A/D)	nl	*B-intense* (2S)	5′ (−419)	128	56	−45.6	TAA TAA	29
Sorghum								
Sb1 (B)	dTIR	HPRG	3′ (+1479)	229	69	−43.5	TAA TAA	30
Sb2 (B)	*Sb1*	HPRG	5′ (−842)	232	70	−39.0	TAT TGG	30
Sb3 (B)	*Sb1*	HPRG	5′ (−598)	248	68	−49.1	TAA ATA	30
Sb4 (B)	*Sb1*	NMDP II	5′ (−868)	242	66	−37.6	—A TAA	31
Sb5 (B)	*Sb1*	PEPC CP21	5′ (−424)	415*	ND	ND	— TCC	32
Sb6 (C)	*Sb1*	PEPC CP46	in 4 (+2246)	343	68	−74.0	TAA TAA	17
Sb7 (B)	dTIR	tRNA-glycine	3′ (+82)	28*	ND	ND	TTT —	33
Rice								
Os1 (C)	*Sb1*	Phytocrome 18	in 5 (+4056)	338	68	−84.6	GAG GAG	18
Os2 (C)	*Sb1*	rab16B	5′ (−650)	330	66	−77.2	TAT TCA	34
Os3 (C)	*Os1*	α-amylase 2A	5′ (−375)	206*	ND	ND	— CAT	35
Os4 (C)	*Os2*	Oryzacystatin-II	in 1 (+244)	299	70	−50.1	TAA TAA	36
Os5 (C)	*Os1*	PAL	5′ (−601)	213*	ND	ND	AAC —	37
Os6 (C)	*Os1*	Starch branching	5′ (−813)	335	70	−82.9	AGT AGC	38
Barley								
Hv2 (D)	*Zm18*	Chalcone synthase	in 1 (+891)	168	64	−48.3	TAA TAA	39

In the *Tourist* name column, the subfamily designation is given in parentheses. Classification of *Tourist-Sb5*, *-Sb7*, *-Os3*, and *-Os5* is tentative. *Tourist-Zm1–11* and *-Hv1* are members of the *Tourist A* subfamily (13). nl, Not listed in databases. A *Tourist* element (*Tourist-Zm23*) is located in the 5′ flanking sequence of the maize *B-intense* gene (V. Chandler, personal communication). *Tourist-Zm22* has been previously referred to as MISD-1 (28). Abbreviations of gene names are as follows: *Cat*, catalase; *Bz*, UDPglucose:flavanol 3-*O*-glucosyltransferase; *Adh*, alcohol dehydrogenase; HPRG, hydroxyproline-rich glycoprotein; NMDP II, NADP-malate dehydrogenase; rab, responsive to abscissic acid; PAL, phenylalanine ammonia-lyase. Chromosome location is indicated in parentheses. Distance between the translation start site of the host gene and the *Tourist* element sequence is given as the position; 5′, 3′, and "in" indicate positions (nt) within the 5′ flanking, 3′ flanking, and intron sequences, respectively. For $\Delta G°$, optimal folding of DNA sequences was performed using the FOLD program (GCG) as described (13). ND, not determined. Inverse orientations were used for flanking sequences of *Tourist-Zm18*, *-Zm19*, *-Zm20*, *-Zm21*, *-Zm22*, and *-Hv2*. —, Sequence not available.

*Only partial sequence available. *Tourist-Sb5* harbors another inverted-repeat element named *Stowaway* (unpublished data).

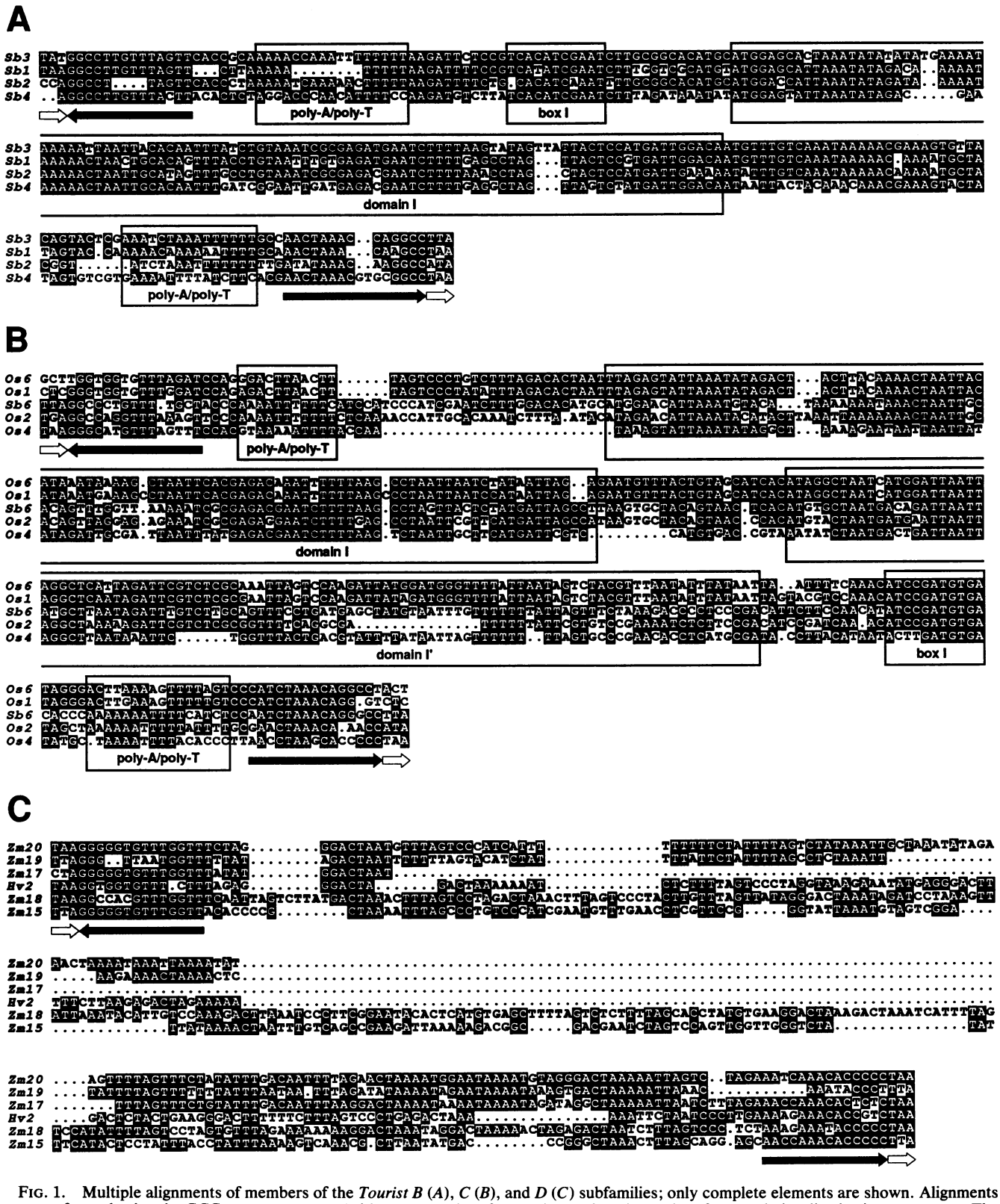

FIG. 1. Multiple alignments of members of the *Tourist B* (*A*), *C* (*B*), and *D* (*C*) subfamilies; only complete elements are shown. Alignments were performed using the GCG program PILEUP (with a gap penalty of 3.0 and a gap length penalty of 0.3) and visualized using BOXSHADE. The inverse orientations of *Tourist-Sb2*, *-Sb3*, *-Sb6*, *-Os1*, *-Os2*, *-Zm18*, and *-Hv2* were used to obtain optimal alignment. Solid arrows indicate the approximate position of the TIRs and open arrows indicate the flanking direct repeats.

distinctive but related TIR sequence and their variable length (Fig. 1*C*).

Multiple Elements Associated with Three Genes. Three *Tourist B* elements are in the flanking DNA of the sorghum hydroxyproline-rich glycoprotein gene (Table 1 and Fig. 2). *Sb2* and *Sb3* are arranged in tandem in the 5′ flanking region with the 3′ terminus of *Sb2* and the 5′ terminus of *Sb3* overlapping by 5 bp. Members of different *Tourist* subfamilies were also found near each other. *Zm5* (*Tourist A*) and *Zm18* (*Tourist D*) are 86 bp apart in the 3′ flanking sequences of the maize oleosin gene. An even more striking example is the two complete (*Zm20*, *Tourist D*; *Zm3*, *Tourist A*) and five

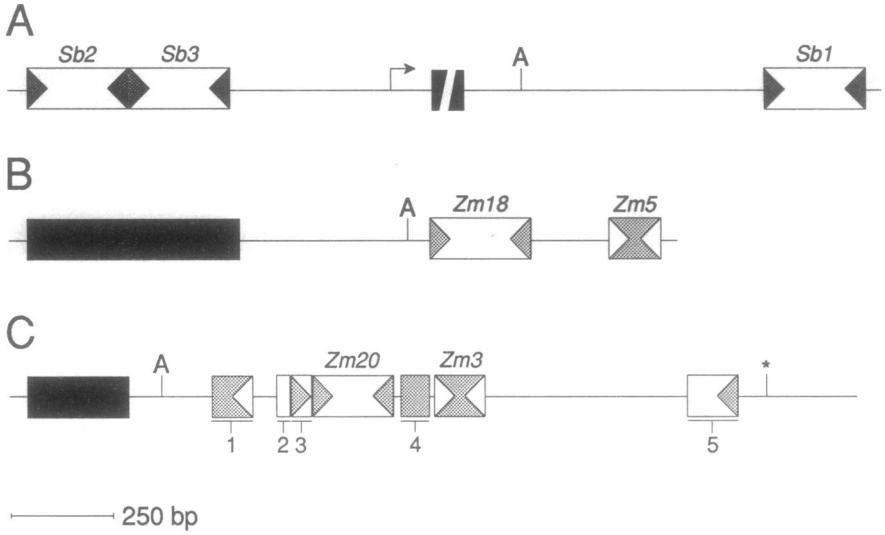

FIG. 2. Complete and partial *Tourist* elements in the flanking regions of the sorghum hydroxyproline-rich glycoprotein gene (*A*) and maize oleosin (*B*) and *Adh1-C*^{m} genes (*C*). *Tourist* element locations are shown relative to coding regions (solid box), transcription start sites (bent arrow), and polyadenylylation signals (*A*). Element subfamilies are indicated by shaded boxes with open inverted arrows (*Tourist A*), open boxes with dark shaded inverted arrows (*Tourist B*), and open boxes with light shaded inverted arrows (*Tourist D*). Inverted arrows indicate TIRs and are not drawn to scale. Boxes corresponding to partial elements (boxes 1–5) in the 3′ flanking region of the maize *Adh1-C*^{m} allele are shaded to indicate their *Tourist* subfamily affiliation. The insertion site of *Tourist-Zm8* in the maize *Adh1-1S* allele is marked by an asterisk.

partial *Tourist* elements in the 3′ flanking region of the maize *Adh1-C*^{m} gene. These partial elements correspond to degenerate *Tourist A* and *D* elements. Fragments 1, 4, and 5 share >60% sequence similarity to *Tourist-Zm1*, *-Zm14*, and *-Zm20*, respectively. The 5′ half of fragment 3 is identical to the first 24 bp of *Zm20*. The remainder of fragment 3 is identical to fragment 2. In total, *Tourist* sequences account for ≈60%, 65%, and 35% of the reported 3′ flanking regions of the sorghum PEPC, maize oleosin, and maize *Adh1-C*^{m} genes, respectively (data not shown).

Mobility of *Tourist A*, *C*, and *D* Subfamilies. *Tourist* was originally identified as a recent insertion into the waxy gene of maize (13). In that study we cited polymorphisms between nonmutant maize genes to document the insertion of two other members of the *Tourist A* subfamily, *Zm3* in *Adh1-C*^{m} and *Zm8* in *Adh1-1S*. Similarly, *Zm20*, a *Tourist D* element located in the 3′ flanking region of maize *Adh1-C*^{m}, corresponds to an insertion polymorphism previously noted between the *Adh1-C*^{m} and *Adh1-1S/F* alleles (27). The *Adh1-1S/F* alleles lack *Zm20* and have only one copy of the target site, 5′-TAA-3′. The determination of the divergence date of *Adh1-C*^{m} and *Adh1-1S* as ≈1.2 million years ago provides evidence for the relatively recent insertion of *Zm3* (*Tourist A*) and *Zm20* (*Tourist D*) (40).

To establish a recent mobile history for *Tourist C* members, *Sb6* and *Os1*, located in the sorghum PEPC and rice phytochrome 18 genes, respectively, were used for a PCR-based search of insertion polymorphisms in homologous loci of related species. These elements were chosen for this analysis because (*i*) both are located in introns (see Table 1), thereby facilitating the selection of PCR primers in the highly conserved flanking exons, and (*ii*) both are flanked by perfect 3-bp direct repeats, thus, allowing us to predict that alleles lacking these elements should have only one copy of the 3-bp target sequence.

As shown in Fig. 3, two subspecies of *Sorghum bicolor* lack *Sb6* from intron 4 of their PEPC genes. Similarly, two wild relatives of the domesticated *Oryza sativa*, *Oryza puncata* and *Oryza eichingeri*, lack *Os1* in intron 5 of their phytochrome 18 genes. In both cases, alleles lacking these elements had only one copy of the putative 3-bp target site.

DISCUSSION

The diversity of the *Tourist* family precludes our ability to use traditional means, such as Southern or slot blots, to estimate element copy number in cereal grass genomes. Pairwise alignments of *Tourist* elements within the same organism indicate that <70% nucleotide similarity is not uncommon (data not shown). Given this level of sequence divergence, even low-stringency washes would not allow cross-hybridization between many of the elements described in this study. Despite this limitation, we believe that the frequent association of single and multiple *Tourist* elements with normal genes of four grasses indicates that this is a very large family that can account for a significant fraction of the repetitive DNA component of these species.

Our findings have important implications concerning the organization and evolution of the genomes of grasses, in particular, and possibly of higher plants in general. A large fraction of the genomes of most higher plants is repetitive. The maize and barley genomes, for example, are ≈78% and 76% repetitive, respectively (41). To understand the structure

A

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Sbb  ATCTTTCAATTAA<-Sb6->TAAGAGAAAAACA
Sbd  ATCTTTCAATTAA          GAGAGAAACA
Sba  ATCTTTCAATTAA          GAGAGAAACA
```

B

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Os   TGTTTTGCTTGAG<-Os1->GAGTCCACTGCTG
Op   GGTTTTGCTTGAG          CCCACTGCTG
Oe   GGTTTTGCTTGAG          CCCACTGCTG
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FIG. 3. DNA sequence of insertion polymorphisms corresponding to *Tourist-Sb6* and *-Os1*. (*A*) *Tourist-Sb6* is located within intron 4 of the PEPC gene from *Sorghum bicolor* spp. *bicolor* (*Sbb*). *Sorghum bicolor* spp. *durra* (*Sbd*) and *Sorghum bicolor* spp. *arundinaceum* (*Sba*) lack *Tourist-Sb6* and have only one copy of the target site 5′-TAA-3′ (underlined). (*B*) Similarly, *Tourist-Os1* is located within intron 5 of the rice phytochrome 18 gene from *Oryza sativa* (*Os*). *Oryza puncata* (*Op*) and *Oryza eichingeri* (*Oe*) lack *Tourist-Os1* and have only one copy of the target site 5′-GAG-3′ (underlined).

of the repetitive DNA of plant genomes, several researchers used hydroxyapatite chromatography and electron microscopy to determine that a large fraction is composed of short, highly repetitive, interspersed sequences that contain inverted repeats (for review, see refs. 42–44). The *Tourist* family fulfills these criteria and as such may represent the prototype element family of inverted repeat interspersed repetitive DNA.

Tourist shares some features with short interspersed elements (SINEs) (45). Like mammalian *Alu* elements, *Tourist* elements are short, mobile, nonautonomous, and frequently associated with genes. In addition, like *Alu*, *Tourist* elements are conserved in distantly related species. It has been estimated that *Alu* elements are present in species that diverged >65 million years ago (46). Similarly, the presence of *Tourist* in the genomes of maize and rice indicates conservation over 50 million years ago (40). These similarities are superficial and are not indicative of an underlying relationship between *Tourist* and *Alu* elements. Rather, their common features reflect the success of these families in their respective hosts.

We are unable, at this time, to classify the *Tourist* family with regard to its mechanism of transposition. Like elements that transpose via a DNA intermediate, it has short TIRs (9, 11). Unlike many DNA elements, however, there is no evidence for the excision of *Tourist* elements in either germinal or somatic lineages. *Tourist* elements lack both an A-rich region at the 3′ terminus and a polymerase III promoter. These features, which are found in short interspersed elements (SINEs), like *Alu*, are commonly associated with RNA-mediated transposition (45). Finally, it is unlikely that *Tourist* elements are "solo" LTRs like those found in several eukaryotes (10, 47, 48). It would be difficult to envision solo LTRs with the structural characteristics of the *Tourist* family. That is, solo LTRs are typically flanked by 5-bp direct repeats, have short TIRs (≈5 bp), and usually do not have significant potential to form stable DNA secondary structures. Our difficulty in classifying *Tourist* at this time may also reflect an unusual mode of transposition. Additional clues to the mechanism of transposition of this interesting and evolutionarily important family may come from experiments to identify either a larger autonomous element or intermediates in the transposition reaction.

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1. Britten, R. J. & Kohne, D. E. (1968) *Science* **161,** 529–540.
2. Flavell, R. B. (1984) *BioEssays* **1,** 21–22.
3. Flavell, R. B. (1986) *Phil. Trans. R. Soc. London B* **312,** 227–242.
4. Vedel, F. & Delseny, M. (1987) *Plant Physiol. Biochem.* **25,** 191–210.
5. Smyth, D. R. (1991) *Chromosoma* **100,** 355–359.
6. Deininger, P. L., Batzer, M. A., Hutchison, C. A. & Edgell, M. H. (1992) *Trends Genet.* **8,** 307–311.
7. Edwards, A., Voss, H., Rice, P., Civitello, A., Stegemann, J., Schwager, C., Zimmermann, J., Erfle, H., Caskey, C. T. & Ansorge, W. (1990) *Genomics* **6,** 593–608.
8. Bennett, M. D. & Smith, J. B. (1991) *Phil. Trans. R. Soc. London B* **334,** 309–345.
9. Weil, C. F. & Wessler, S. R. (1990) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41,** 527–552.
10. Grandbastien, M.-A. (1992) *Trends Genet.* **8,** 103–108.
11. Gierl, A. & Saedler, H. (1992) *Plant Mol. Biol.* **19,** 39–49.
12. Leeton, P. R. J. & Smyth, D. R. (1993) *Mol. Gen. Genet.* **237,** 97–104.
13. Bureau, T. E. & Wessler, S. R. (1992) *Plant Cell* **4,** 1283–1294.
14. Dellaporta, S. L., Wood, J. & Hicks, J. B. (1983) *Plant Mol. Biol. Reporter* **1,** 19–22.
15. Devereaux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
16. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
17. Lepiniec, L., Keryer, E., Tagu, D., Gadal, P. & Crétin, C. (1992) *Plant Mol. Biol.* **19,** 339–342.
18. Kay, S. A., Keith, B., Shinozaki, K. & Chua, N.-H. (1989) *Nucleic Acids Res.* **17,** 2865–2866.
19. Vieira, J. & Messing, J. (1987) *Methods Enzymol.* **153,** 3–11.
20. Abler, M. L. & Scandalios, J. G. (1993) *Plant Mol. Biol.* **22,** 1031–1038.
21. De Framond, A. J. (1991) *FEBS Lett.* **290,** 103–106.
22. Zhang, R. & Walker, J. C. (1993) *Plant Mol. Biol.* **21,** 1171–1174.
23. Furtek, D., Schiefelbein, J. W., Johnston, F. & Nelson, O. E. (1988) *Plant Mol. Biol.* **11,** 473–481.
24. Athma, P., Grotewold, E. & Peterson, T. (1992) *Genetics* **131,** 199–209.
25. Qu, R. & Huang, A. H. C. (1990) *J. Biol. Chem.* **265,** 2238–2243.
26. Christensen, A. H., Sharrock, R. A. & Quail, P. H. (1992) *Plant Mol. Biol.* **18,** 675–689.
27. Osterman, J. C. & Dennis, E. S. (1989) *Plant Mol. Biol.* **13,** 203–212.
28. Zack, C. D., Ferl, R. J. & Hannah, L. C. (1986) *Maydica* **31,** 5–16.
29. Radicella, J. P., Brown, D., Tolar, L. A. & Chandler, V. L. (1992) *Genes Dev.* **6,** 2152–2164.
30. Raz, R., Crétin, C., Puigdomènech, P. & Martínez-Izquierdo, J. A. (1991) *Plant Mol. Biol.* **16,** 365–367.
31. Luchetta, P., Crétin, C. & Gadal, P. (1991) *Mol. Gen. Genet.* **228,** 473–481.
32. Lepiniec, L., Keryer, E., Philippe, H., Gadal, P. & Crétin, C. (1993) *Plant Mol. Biol.* **21,** 487–502.
33. Ma, D.-P. & Yang, Y.-W. (1988) *Nucleic Acids Res.* **16,** 3588.
34. Yamaguchi-Shinozaki, K., Mundy, J. & Chua, N.-H. (1989) *Plant Mol. Biol.* **14,** 29–39.
35. Huang, N., Reinl, S. J. & Rodriguez, R. L. (1992) *Gene* **111,** 223–228.
36. Kondo, H., Abe, K., Emori, Y. & Arai, S. (1991) *FEBS Lett.* **278,** 87–90.
37. Minami, E. & Tanaka, Y. (1993) *Biochim. Biophys. Acta* **1171,** 321–322.
38. Kawasaki, T., Mizuno, K., Baba, T. & Shimada, H. (1993) *Mol. Gen. Genet.* **237,** 10–16.
39. Rohde, W., Dörr, S., Salamini, F. & Becker, D. (1991) *Plant Mol. Biol.* **16,** 1103–1106.
40. Gaut, B. S. & Clegg, M. T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2060–2064.
41. Flavell, R. B., Bennett, M. D., Smith, J. B. & Smith, D. B. (1974) *Biochem. Genet.* **12,** 257–269.
42. Flavell, R. (1980) *Ann. Rev. Plant Physiol.* **31,** 569–596.
43. Flavell, R. B. (1982) in *Nucleic Acids and Proteins in Plants II*, eds. Parthier, B. & Boulter, D. (Springer, Berlin), pp. 46–74.
44. Flavell, R. B. (1985) in *Genetic Flux in Plants*, eds. Hohn, B. & Dennis, E. S. (Springer, Berlin), pp. 139–156.
45. Deininger, P. L. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 619–636.
46. Deininger, P. L. & Daniels, G. R. (1986) *Trends Genet.* **2,** 76–80.
47. Boeke, J. D. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 335–374.
48. Robins, D. M. & Samuelson, L. C. (1992) *Genetica* **86,** 191–201.